

PRELIMINARY AMENDMENT  
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Page 2, lines 1-22, delete in their entirety, and insert therefor

-- By the way, active oxygen is important as a biological protection factor such as bactericidal action of leucocytes, but it becomes apparent that the excessive production of active oxygen in the living body causes various tissue disorders.

As the ordinary factors for generating active oxygen, there have been known stresses, alcohols, peroxides, drugs, exercises and the like. It is pointed out that active oxygen and lipoperoxide generated by these factors are closely connected with cerebral nerve diseases, circulatory diseases, cancers, alimentary diseases, hepatic diseases, arterial sclerosis, renal diseases, diabetes, aging and the like.

a2 The living body retains a series of oxidation protection systems so to protect itself against oxygen toxicity. On the other hand, in order to permit these systems to function normally, it is important to ingest oxidation nutrient components sufficiently. As the natural oxidation nutrient components, there have been known vitamin E, vitamin C, s-carotene, polyphenol and trace elements (e.g. selenium, copper, zinc, etc.). For the purpose of affording an antioxidation action, food products containing these nutrient components have been developed. --

Page 2, lines 23-25, delete in their entirety.

Page 3, lines 1-7, delete in their entirety, and insert therefor

a3 -- In vivo antioxidation mechanism is classified roughly into a preventive antioxidation action (controlling the generation of a radical) and a linkage-breakage type antioxidation action (scavenging and eliminating a radical which has already been

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a3  
cont  
generated) according to its action. Examples of those which have the former action include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and the like. Examples of those which have the latter action include the above antioxidation nutrient components. --

Page 3, lines 12-18, delete in their entirety, and insert therefor

100237954  
a4  
-- It is an object of the present invention to provide an antioxidation food product, an antioxidation preparation and an antioxidation method for the linkage-elimination of superoxide and hydrogen peroxide, which express a superoxide dismutase (hereinafter referred to as "SOD")-like activity and a catalase (hereinafter referred to as "CAT") activity, simultaneously, and which are particularly superior in preventive antioxidation action. --

Page 3, lines 19-25, delete in their entirety, and insert therefor

a5  
-- The antioxidation food product of the present invention has an antioxidation action in the living body, comprising a fermented product produced by adding a manganese-containing natural material and fermenting with bacteria having a CAT activity. By "living body" is meant the physical body of living, multicellular organisms including, but not limited to humans and other mammals. --

Page 4, lines 1-19, delete in their entirety, and insert therefor

a6  
-- That is, the present inventors have obtained a knowledge that specific bacteria among various lactic acid bacteria express no CAT activity under the environment in which manganese is not present, but if manganese is present in the growing

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environment, they incorporate manganese into their cells to express a Mn-CAT activity and a SOD-like activity, simultaneously. The antioxidation food product of the present invention is particularly suitable as a preventive antioxidation food product.

It is known that Mn-CAT is not affected by various inhibitors, modifiers, chelating agents, etc. in comparison with heme-CAT containing iron, and exhibits stability within a wide range of pH and temperature.

Further, the antioxidation food product of the present invention may be a dried product, preferably a freeze-dried product which contains bacteria having a CAT activity and a manganese-containing natural material, in addition to the fermented product as described above by "manganese-containing natural material" is meant any material whether naturally occurring or synthetically produced which contains the element manganese bound to another organic or inorganic chemical moiety. The term is not meant to include elemental manganese. --

**Page 4, line 25, delete in its entirety.**

**Page 5, lines 1-10, delete in their entirety, and insert therefor**

-- Furthermore, according to the present invention, there is provided an antioxidation method in the living body including the interior of a digestive tract, which comprises ingesting a fermented product produced by adding a manganese-containing natural material to a food product and fermenting with bacteria having a CAT activity to express a SOD-like activity and a CAT activity, simultaneously.

a1  
cont

activity and CAT activity due to the addition of  $\text{MnCl}_2$  or tea. --

Page 7, lines 4-14, delete in their entirety, and insert therefor

28  
TOTAL: 92000

-- Recently, the clinical application of SOD against 5 rheumatism, cardiac ischemia, etc. have been proceeded. However, there is a serious problem that the local amount of hydrogen peroxide is increased when SOD is used alone and, further, a hydroxy radical ( $\cdot\text{OH}$ ) having a highest radical reactivity is formed. Accordingly, it is important to eliminate  $\text{O}_2^-$  and remove hydrogen peroxide by using CAT and GSH-Px in combination when employing SOD. Therefore, for the above reason, the coupling action between SOD-like and CAT obtained by adding Mn to *Lactobacillus plantarum* is considered to be very important. --

important. --

Page 8, lines 9-22, delete in their entirety, and insert therefor

-- The manganese-containing natural material in the present invention is used for supplying manganese to the bacteria cells. It is impossible to add manganese as an inorganic compound because manganese itself is not accepted as a food additive and, therefore, the natural material was used. Examples of the natural material containing a large amount of manganese include plants such as teas, vegetables (e.g. represented by cabbage, spinach, etc.), herbs and the like. Accordingly, it is important to add these plants so as to supply manganese which is necessary for expressing a CAT activity and a SOD-like activity to bacteria having a CAT activity. Particularly, since the tea contains a lot of antioxidation components such as catechin,

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99  
cont vitamin C, various trace elements, etc., additional effects can be obtained by taking advantage of the presence of an activity of these anti-oxidant components."

Page 8, lines 23-25 delete in their entirety.

Page 9, lines 1-5, delete in their entirety, and insert therefor

210  
-- Particularly, the addition of the tea to *Lactobacillus plantarum* causes the following effect, that is, not only the SOD-like activity and CAT activity are expressed but also the SOD-like activity is extremely high in comparison with the case using an inorganic Mn compound (e.g. manganese chloride  $MnCl_2$ , etc.) and, further, the SOD-like activity and CAT activity under gastric juice exposure can be maintained for a long period of time. --

Page 9, lines 6-21, delete in their entirety, and insert therefor

sub B2  
OK -- It is preferred that the tea or the other natural material is added to a food product in the form of powder. The incorporation of manganese due to bacteria is facilitated if the magnesium source is a powder. The pulverization is conducted by extracting a natural material with water and/or water-miscible organic solvent (e.g. alcohols such as ethyl alcohol, etc.) and then with an organic solvent which is in-miscible to water (e.g. chloroform, ethyl acetate, butanol, etc.) to separate into an organic phase and an aqueous phase, recovering a dissolved solid content from the aqueous phase, followed by drying. Further, a solid content may also be recovered from a solution extracted with water and/or water-miscible organic solvent and then pulverized. Furthermore, an aqueous solution prepared by extracting the natural material with water may be added without

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Sub B1  
Cant  
a11  
pulverizing, or ~~the~~ pulverized product of the natural material  
may be added as it is. --

Page 10, lines 11-13, delete in their entirety, and insert  
therefor

a12  
-- Typical examples of the form of the antioxidation food  
product of the present invention include fermented food products  
and (freeze)dried food products, as described above. --

Page 11, lines 6-23, delete in their entirety, and insert  
therefor

a13  
-- Further, a lactic acid bacteria beverage can be obtained by  
adding a predetermined amount of a manganese-containing natural  
material to a mixed solution of skim milk and sugar (e.g.  
glucose, sucrose, etc.), inoculating the mixed solution with  
lactic acid bacteria having a CAT activity and fermenting at  
35 to 37 °C for about 12 to 72 hours. Yogurt (e.g. liquid  
yogurt type, juice type, etc.) can be produced according to a  
proportion of skim milk to a diluting solution (e.g. water,  
fruit juice, etc.). As the base to be fermented, for example,  
there can be used milk serum, low fat milk, etc., in addition to  
skim milk. Examples of the diluting solution include flesh,  
lactocoffee, etc., in addition to water.

The dried product in the present invention is obtained by  
mixing about  $5 \times 10^8$  to  $5 \times 10^{10}$  bacteria cells 20 with 2 to 4 g  
of a manganese-containing natural material, adding an excipient  
to the mixture, followed by drying. Examples of the excipient  
include lactose, glucose, sucrose, oligosaccharide and the  
like. --

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Page 13, lines 4-8, delete in their entirety, and insert therefor

a14  
-- As shown in Fig. 1, the SOD-like activity increased in proportion to the amount of Mn to be added in both cases (addition of  $MnCl_2$ /tea), but the absolute value of the SOD-like activity is about 35 times greater when tea is added than when 200 M of the Mn is added. --

Page 13, lines 14-15, delete in their entirety, and insert therefor

ab  
-- The function of SOD and CAT is the elimination of  $O_2^-$  and  $H_2O_2$ . However, SOD and CAT are enzymes and, therefore, it is said that SOD and CAT are devitalized even if they are orally ingested and no effect is obtained. However, since the SOD activity and CAT activity are retained in the bacteria cells in case of the Mn-added product produced by fermenting with *Lactobacillus plantarum*, it is expected that these activities are retained until the bacteria are killed. Further, as described above, since Mn itself and the component in the tea have a SOD-like activity but they are not enzymes, it is considered that they are not easily devitalized. --

Page 14, lines 11-20, delete in their entirety, and insert therefor

a16  
-- Immediately after ligating the pylorus of SD male rats (weight: 250 g), 5 ml of a product produced by fermenting with *Lactobacillus plantarum* was administered using an oral probe and contents in the stomach were recovered with time to examine a change in SOD-like activity and CAT activity. The results are shown in Figs. 2 and 3. Further, a test product was obtained by adding  $MnCl_2$  or a tea (powdered green tea) in the concentration (concentration of Mn) of 50  $\mu M$  to an APT broth and, after

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Calb  
Cont inoculating with *Lactobacillus plantarum*, fermenting for  
16 hours. --

Page 19, lines 11-17, delete in their entirety, and insert  
therefor

017  
-- As the product produced by fermenting with  
*Lactobacillus plantarum*, were obtained by adding  $MnCl_2$  or a tea  
extract solution (those obtained by extracting 100 g of a green  
tea with 1 liter of hot water) to an APT broth in the  
concentration (concentration of Mn) of 50  $\mu M$  and, after  
inoculating with *Lactobacillus plantarum*, fermenting for  
16 hours. --

Page 22, lines 20-25, delete in their entirety.

Page 23, lines 1-6, delete in their entirety, and insert  
therefor

sub 82  
9/8  
-- It has been known that the neutrophil produces active  
oxygen so as to detoxify foreign materials and bacteria in the  
living body. Particularly, the neutrophil has a dimutated  
system of hydrogen peroxide which is referred to as "MPO",  
thereby producing perchloric acid or monochlor amine having high  
cytotoxicity. At the time of the excessive reaction, the  
transudation of the neutrophil to the exterior of the neutrophil  
is confirmed and it is considered to be one factor to cause the  
disorder of the biomembrane. There is also a report that the  
infiltration of neutrophil is connected with its crisis  
mechanism in indomethacin-induced gastric ulcer and hydrogen  
peroxide-induced gastric mucosal lesions. --

IN THE CLAIMS:

✓ Please cancel Claims 1-6.